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TRANSPLACENTAL TRANSFER OF ORGANOCHLORINES IN CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*)

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Abstract—The transplacental transfer of organochlorines (OCs) in California sea lions (*Zalophus californianus*) was investigated by analyzing blubber samples from 20 female sea lions and their fetuses during the last trimester of pregnancy. A rapid, high-performance liquid chromatographic, photodiode-array method was used to measure blubber concentrations of polychlorinated biphenyls (PCBs), including dioxin-like congeners, as well as DDTs and hexachlorobenzene. Summed values of PCBs (ΣPCBs), of DDTs (ΣDDTs), and of PCB toxic equivalents (ΣPCB TEQs) were calculated from these data. The ratios of mean blubber concentrations of fetal ΣPCBs to maternal blubber concentrations of ΣPCBs were 0.45 by wet weight and 0.97 by lipid weight, but these ratios varied widely among mother–fetus pairs. Mean ratios of fetal ΣDDTs to maternal ΣDDTs were 0.53 by wet weight and 1.12 by lipid weight. Fetuses were classified into two age groups, based on date of recovery, to examine differences in OC transfer because of gestational age. Fetal to maternal ratios for individual PCB congeners, DDT compounds, and ΣPCBs, ΣDDTs, and ΣPCB TEQs were lower among premature compared with late-term fetuses. These ratios increased for both groups as the logarithmic *n*-octanol/water partition coefficient (log K_{OW}) for each compound decreased. Linear predictions for ΣPCB and ΣDDT concentrations in fetal blubber could be obtained using the ΣPCB and ΣDDT concentrations in maternal blubber, maternal and fetal blubber lipid content, maternal mass, and maternal age. Fetal TEQ was explained by maternal TEQ and maternal age. The ability to predict contaminant concentrations in fetal blubber from maternal parameters is important for developing risk assessment models for marine mammals.

Keywords—California sea lion DDTs Organochlorines Fetus Placenta

INTRODUCTION

Organochlorine (OC) compounds, such as polychlorinated biphenyls (PCBs) and DDTs, are persistent, lipophilic pollutants that biomagnify in marine food webs, with highest concentrations frequently found in top-predator homeotherms, such as marine mammals [1]. Experimental exposure studies in harbor seals (*Phoca vitulina*) demonstrated that these chemicals were associated with decreased circulating vitamin A and thyroid hormone concentrations [2], impaired immune function [3], and lowered reproductive output [4]. Furthermore, studies of free-living animals have linked high blubber concentrations with a higher occurrence of infectious disease and potential endocrine-disrupting effects in a variety of marine mammal species [5–7].

Various intrinsic as well as extrinsic factors can influence the amount of OCs found in an individual at a particular life stage. Most notable are the differences between the sexes in adulthood. Studies reporting blubber concentrations of OCs in marine mammals have regularly demonstrated lower levels of PCBs and DDTs in adult females compared with levels in adult males [1]. This difference is largely because of the transfer of contaminants from mother to offspring during gestation and lactation. A number of studies have shown that lactation is the major route for this transfer of OCs because of the assimilation of maternal blubber and associated OCs into milk [8–10]. Studies regarding the transplacental transfer of these compounds in marine mammals during the gestational period, however, are extremely limited. This lack of data is of concern, because prenatal exposure of the mammalian fetus to PCBs and DDTs can impair fetal development [11,12], and it has been associated with an increased risk of intrauterine fetal growth retardation in humans [13]. This information also is critical for population-level models of risk assessment [14] and for our understanding of the movements, distribution, and fate of OCs in highly exposed species, such as marine mammals. Measurements of OC levels in umbilical cords and maternal blood have been performed in humans [15,16], but for marine mammals, such data are exceptionally rare. Tissue levels of OCs have been reported in a single pregnant striped dolphin (*Stenella coeruleoalba*) and her fetus [17], a single harbor porpoise (*Phocoena phocoena*) fetus [18], and 11 pilot whale (*Globecephala melas*) fetuses [19]. Organochlorines also were measured in the matched liver samples from five mother–fetus pairs of gray seals (*Halichoerus grypus*) [20]. To our knowledge, however, no data have been published regarding the transplacental transfer of contaminants in otariids.

California sea lions (*Zalophus californianus*) are exposed to some of the highest levels of contaminants worldwide because of their geographical range and trophic position [1]. During the early 1970s, the high blubber levels of DDTs and PCBs in California sea lions from San Miguel Island (CA, USA) were associated with premature pupping and stillbirths

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[21]. The role of these OCs in the etiology of this reproductive failure, however, remains unclear because of the confounding effects of factors not controlled for in that study, including maternal transfer of OCs to the developing fetus [22]. Other health effects in this species also have been linked to OC exposure, such as a high prevalence of cancer [23]. The associations between reproductive failure and cancer and contaminant exposure in sea lions remain equivocal, partially as a result of the complexities of the dynamics of OC levels in blubber; sampling at a given time point for exposure assessment may result in biased estimates because of the influence of transplacental and lactational transfer as well as the effects of mass loss on maternal blubber levels.

The present study investigates the transplacental transfer of PCBs and DDTs in California sea lions by comparing concentrations of these contaminants in the blubber of 20 fetuses and their mothers stranded in central California with acute domoic acid toxicosis. Fetus to mother partition ratios are examined in two fetal age groups to determine whether the transfer of PCB congeners changes over the course of gestation. Finally, it examines how predictable blubber OC concentrations are in the fetus using body condition parameters of the fetus and OC concentrations, life history, and condition variables of the mother. This information will assist future population-level risk assessment studies that must take into account transplacental transfer rates [14], particularly when reproductive endpoints are being modeled that affect offspring survival and, therefore, population dynamics.

MATERIALS AND METHODS

Sea lion sampling

Blubber samples were collected from adult female California sea lions ($n = 20$) stranded along the California coast from 1998 to 2002 and their fetuses. All adults stranded because of domoic acid intoxication, as determined by specific neurological symptoms (e.g., seizures, ataxia, head weaving, and abnormal scratching) and were in good body condition [24]. No other disease processes beyond domoic acid were detected at clinical or postmortem examination [24,25]. Blubber from live adults was collected with a sterile, 8-mm biopsy punch after manual restraint, local administration of lidocaine, and intramuscular administration of midazolam (Versed; Roche Laboratories, Nutley, NJ, USA) at 0.2 mg/kg. Blubber from dead animals was collected during necropsy using sterile scalpel blades. Samples from live and dead animals included the full thickness of the blubber from skin to muscle. Samples were collected over the sternum in dead animals and between the scapulae in live animals ($n = 15$ dead and 5 live adult females). Fetuses ($n = 20$) were sampled as described above either postmortem from dead pregnant females or following euthanasia because of poor prognosis after premature birth from live animals. Blubber was stored in solvent-rinsed Teflon® sheets and frozen at −20°C until analysis. Standard body length, mass, and ventral blubber thickness were measured on each dead animal, and maternal age was determined by counting the dentinal growth layers on the cut surface of the upper left canine collected at necropsy as described by Greig et al. [25]. Because the implantation date was not known, it was not possible to estimate the age of the fetus in terms of days. Therefore, the Julian day at which the fetus was recovered (either during necropsy or after the fetus was aborted) was used to approximate the stage of fetal development. Mean pupping date for full-term sea lion fetuses is Julian day 166, or June 15 [21].

Organochlorine and lipid analysis

Blubber samples were analyzed for selected OCs, including dioxin-like coplanar PCBs and DDTs, by a rapid, high-performance liquid chromatographic, photodiode-array (HPLC/PDA) method [25]. Using this method, concentrations of dioxin-like PCB congeners as well as concentrations of other PCB congeners, DDTs, and hexachlorobenzene were measured, and summed values of PCBs (ΣPCBs), of PCB toxic equivalents (ΣPCB TEQs), and of DDTs (ΣDDTs) were calculated from these data. Previous studies have shown that the OC concentrations determined by the HPLC/PDA method are in good agreement with the values measured by other, more frequently used analytical methods (e.g., gas chromatography/electron-capture detection and gas chromatography/mass spectrometry) for a wide range of marine biota matrices, including marine mammal blubber [26,27]. Briefly, blubber (0.2–0.3 g), hexane/pentane (1:1, v/v), sodium sulfate (5 g), and a surrogate standard (1,2,3,4-tetrachlorodibenzo-*p*-dioxin; 250 ng) were homogenized. The OCs were separated from interfering compounds (e.g., lipids and aromatic compounds) in the sample extracts by elution with hexane/methylene chloride (1:1, v/v) on a gravity-flow cleanup column that contained neutral, basic, and acidic silica gels. Before the cleanup step, a 1-ml aliquot of each sample extract was removed for lipid quantitation. The coplanar, or dioxin-like, congeners (PCBs 77, 105, 118, 126, 156, 157, 169, and 189) were resolved from other selected PCBs (PCBs 101, 110, 128, 138, 153, 170/194, 180, 190, and 200) and chlorinated pesticides (*o,p′*-dichlorodiphenyldichloroethane [DDD], *p,p′*-DDD, *p,p′*-dichlorodiphenyldichloroethylene [DDE], *o,p′*-DDT, *p,p′*-DDT, and hexachlorobenzene) by HPLC on two Cosmosil 2-(1-pyrenyl) ethyl analytical columns (Nacalai Tesque, Kyoto, Japan; purchased through Phenomenex, Torrance, CA, USA) connected in series and cooled to 16°C. The congeners were measured by an ultraviolet PDA and were identified by comparing their ultraviolet spectra (200–310 nm) and retention times to those of reference standards in a library. The analyte purity was confirmed by comparing spectra within a peak to the apex spectrum.

Summed PCBs were calculated by adding the concentrations of PCBs 77, 101, 105, 110, 118, 126, 128, 138, 153, 156, 157, 169, 170/194, 180, 189, 190, and 200 (based on individual response factors) plus concentrations of other PCBs (PCBs 28, 31, 47, 52, 70, 146, and 209) calculated by summing areas of peaks identified as these PCBs and using an average PCB response factor. Summed DDTs were calculated by adding the concentrations of *o,p′*-DDD, *o,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD, and *p,p′*-DDT. Summed PCB TEQs were calculated by multiplying the molar concentration of each dioxin-like PCB congener by the appropriate toxic equivalency factor, as recommended by the World Health Organization for human and wildlife health [28]. The following toxic equivalency factors were used for the PCB TEQ calculations: PCB 77, 0.0001; PCB 105, 0.0001; PCB 118, 0.0001; PCB 126, 0.1; PCB 156, 0.0005; PCB 157, 0.0005; PCB 169, 0.01; and PCB 189, 0.0001. If the concentration of a dioxin-like PCB was less than the limit of detection, a value of zero for the PCB TEQ for that specific congener was used in the calculation to avoid overestimation of toxicity. Although the TEQs determined in the sea lion blubber samples were conservative values, both because they did not include polychlorinated dibenzodioxins

Table 1. Mean, standard deviation (SD), ranges, and sample size (*n*) of maternal and fetal life history variables[a]

	Maternal					Premature fetuses					Late-term fetuses				
	Mean	SD	Min	Max	*n*	Mean	SD	Min	Max	*n*	Mean	SD	Min	Max	*n*
Length (cm)	164	9	144	180	20	59	7	47	69	9	73	2	70	77	11
Mass (kg)	86	15	60	105	20	5	2	2	7	8	7	1	5	10	9
Blubber thickness (mm)	26	9	15	42	14	5	3	2	10	7	8	4	4	14	6
Age (year)	9	2	5	12	15										
Julian date of birth						104	16	67	116	9	147	4	142	152	11
% Lipid	45	18	7	71	20	15	7	6	26	9	24	9	6	34	11

[a] Max = maximum; Min = minimum.

(PCDDs) or polychlorinated dibenzofurans (PCDFs) and because the limit of detection for dioxin-like PCBs for the HPLC/PDA method were higher than those measured by high-resolution gas chromatography/mass spectrometry, they provide an estimate of toxic potency in the samples. Previous contaminant studies have shown that dioxin-like PCBs are greater contributors (usually >80%) to the total TEQs compared to the PCDDs and PCDFs in marine mammals [29–32]. For example, Ross et al. [33] found that dioxin-like PCBs contributed more than 90% to the total TEQs determined in blubber of harbor seal pups from Puget Sound (WA, USA). This is because dioxin-like PCBs frequently are measured in much higher concentrations than are PCDDs or PCDFs in the marine environment, even though they are less toxic than TCDD [28]. The PCB TEQs are reported as pg/g wet weight or pg/g lipid weight.

Lipid content (reported as % lipid) of blubber was measured by thin-layer chromatography/flame-ionization detection (TLC/FID) using an Iatroscan Mark 5 (Iatron Laboratories, Tokyo, Japan) [34,35]. This method of lipid quantitation frequently is used in the food science industry and other scientific disciplines, because it provides information regarding lipid classes as well as percentage lipid content [34,36–39]. Each lipid sample extract was spotted on a Chromarod SIII (Shell-USA, Fredericksburg, VA, USA) and developed in a solvent system containing hexane/diethyl ether/formic acid (60:10:0.02, v/v/v). Various classes of lipids (i.e., wax esters/sterol esters, triglycerides, free fatty acids, cholesterol, and phospholipids/other polar lipids) were separated based on polarity, with the nonpolar compounds (i.e., wax esters/sterol esters) eluting first, followed by the more polar lipids. Data were acquired and analyzed using Waters Millennium software (Waters, Milford, MA, USA). A four-point linear external calibration was used for quantifying each lipid class. Total lipid concentrations were calculated by adding the concentrations of the five lipid classes for each sample and reported as the percentage total lipid. Duplicate TLC/FID analyses were performed for each sample extract, and the mean value was reported.

The HPLC/PDA system was calibrated daily to ensure that it was in optimum operating condition. A method blank and a National Institute of Standards and Technology (Gaithersburg, MD, USA) Standard Reference Material (SRM 1945) blubber sample were analyzed with each sample set (comprised of 12 field samples), and results met laboratory criteria [40]. Approximately 10% of the sea lion blubber samples were analyzed in duplicate to measure the precision of the method, and the laboratory quality-assurance criteria were met for all analytes detected in the blubber samples. Method blanks also met laboratory criteria. The limit of detection for the PCB congeners ranged from less than 0.46 to less than 5.1 ng/g wet weight. The limit of detection for the DDTs ranged from less than 1.4 to less than 9.6 ng/g wet weight.

Analysis of data

For calculating fetus to mother partition ratios of PCB congeners and DDT compounds, only values above the detection limit were used. Partition ratios were plotted against logarithmic *n*-octanol/water partition coefficients (log K_{OW}) obtained from the literature [41–43]. All statistical analyses were carried out using the S-Plus 6.1 software (Insightful, Seattle, WA, USA) and SPSS 11.0 software (SPSS, Chicago, IL, USA). Because they were log-normally distributed, all contaminant data were $\log_{10}$-transformed before analysis. All subsequent statistical analyses (including *t* tests and regression analyses) were carried out using the transformed values. The results of the predictive linear least-squares models (with *F* values from the associated analysis of variance [ANOVA] tables) are reported. Model fits were assessed using residual analysis, and collinearity statistics, including variance inflation factors, were used to determine model appropriateness.

RESULTS

Organochlorine concentrations in females and fetuses

The mean age of the adult females was nine years (n = 15) (Table 1), and all females were probably multiparous based on their age or standard body length and the life history of the California sea lion [25,44]. All fetuses were sampled during the last trimester of pregnancy, and they were classed as premature (Julian date of birth < 120 d) or late term (Julian date of birth > 120 d) (Table 1). Sample collection was associated with two peaks in timing of domoic acid toxicosis events, which influenced the availability of stranded animals for sampling [24]. Despite the nutritionally robust appearance and thick blubber depths measured in the adult females, the lipid content of three of the adults was surprisingly low (7, 12, and 13%). Lipid content also varied among age groups: Adults had greater lipid content than the late-term fetuses, which in turn had greater lipid content than the premature fetuses. Because of this variation in lipid content, both wet-weight and lipid-normalized OC values were used in subsequent analyses: Lipid-normalized values to allow comparison with other published data, and wet-weight values to evaluate the importance of percentage lipid and blubber depth in the predictive regression models. Geometric means and 95% confidence limits were calculated for each of the contaminant groups, because all the concentrations measured in the blubber were log-normally distributed (Table 2). Geometric mean concentrations of ΣDDTs were significantly higher than those of ΣPCBs (on a wet- and

Table 2. Geometric means, 95% confidence intervals, and minimum and maximum concentrations of contaminants measured in blubber samples from adult female and fetal California sea lions[a]

	Geometric mean	Lower geometric CI	Upper geometric CI	Minimum	Maximum	Sample size (*n*)
Mother						
ΣPCBs wet wt	2,757	1,881	4,040	510	18,000	20
ΣPCB TEQs wet wt	42	27	65	7	220	20
ΣDDTs wet wt	7,222	4,341	12,014	630	65,000	20
ΣPCBs lipid wt	7,096	4,883	10,311	1,800	33,000	20
ΣPCB TEQs lipid wt	108	65	179	12	720	20
ΣDDTs lipid wt	18,567	11,785	29,255	4,600	120,000	20
Premature						
ΣPCBs wet wt	757	466	1,231	330	2,900	9
ΣPCB TEQs wet wt	20	11	37	8	110	9
ΣDDTs wet wt	2,006	927	4,342	730	18,000	9
ΣPCBs lipid wt	5,449	3,223	9,211	2,100	16,000	9
ΣPCB TEQs lipid wt	147	81	265	42	420	9
ΣDDTs lipid wt	14,560	6,874	30,839	3,700	69,000	9
Late term						
ΣPCBs wet wt	1,492	798	2,789	350	5,000	11
ΣPCB TEQs wet wt	15	9	27	5	44	11
ΣDDTs wet wt	4,951	2,506	9,781	1,300	20,000	11
ΣPCBs lipid wt	6,831	4,317	10,809	2,300	18,000	11
ΣPCB TEQs lipid wt	70	45	109	23	160	11
ΣDDTs lipid wt	22,738	13,295	38,887	7,500	77,000	11

[a] All concentrations are reported as ng/g on a wet weight (wet wt) or lipid weight (lipid wt) basis except for the summed polychlorinated biphenyl (PCB) toxic equivalents (ΣPCB TEQs), which are reported as pg/g; CI = confidence interval; Σ = summed concentration.

lipid-weight basis; paired *t* tests, $p < 0.0001$) in all samples, and most of the DDT was present as the metabolite *p,p′*-DDE (Table 2). Concentrations of PCBs were less than the limit of detection in all samples for six of the congeners measured in the present study: PCBs 77, 110, 126, 157, 169, and 189. Concentrations of hexachlorobenzene were less than the limit of detection in all blubber samples analyzed in the present study.

Ratio of fetal to maternal contaminant concentrations

For each mother–fetus pair, the ratio of fetal to maternal blubber OC concentration was calculated and the mean values reported. The 2.5th and 97.5th percentiles also were calculated. For the 20 mother–fetus pairs, mean fetal ΣPCB to maternal ΣPCB ratios were 0.45 by wet weight and 0.97 by lipid weight. These ratios varied widely among mother–fetus pairs, with the percentiles ranging up to sixfold. Mean fetal ΣDDT to maternal ΣDDT ratios were 0.53 by wet weight and 1.12 by lipid weight. The mean percentage lipid concentration in sea lion fetus blubber samples was 20% ± 8.8% (mean ± standard deviation), whereas the maternal value was 45% ± 18% (Table 1). Differences between mother to fetus contaminant ratios based on wet weight or lipid weight reflect the low lipid concentration in fetal blubber (Table 1), resulting in ratios greater than 1.0, particularly for ΣDDTs and ΣPCB TEQs. We also calculated ΣDDT to ΣPCB ratios in both fetus and mother to determine if a preferential transfer occurred from mother to fetus for a particular OC group. The mean fetal ΣDDT wet weight to ΣPCB wet weight ratio (3.2) was slightly greater than the mean maternal value (2.8).

Partition ratios of fetal blubber OCs to maternal blubber OCs were calculated for premature and late-term mother–fetus pairs. Mean partition ratios ranged from 0.153 to 1.383 and were lower among the premature group than among the late-term group for every compound measured (Table 3). The greatest mean partition ratios were observed among the DDTs, especially among the late-term group. The mean partition ratio, separated into premature and late-term groups, was plotted against log K_{OW} to illustrate the relationship between partition ratio and hydrophobicity of the different OCs (Fig. 1). The DDTs were the least hydrophobic and had the greatest partition ratio, whereas the more hydrophobic heptachlorinated biphenyls were more resistant to transfer (Fig. 1).

Predicting fetal contaminant levels from physiological variables and maternal OC levels

To determine the most important predictors of fetal contaminant concentrations, a series of least-squares linear models were constructed (using stepwise regression and Akaike's information criterion for model selection [45]). For each dependent variable (fetal ΣPCBs, ΣDDTs, and TEQs), a series of maternal and fetal physiological and contaminant predictors were included as independent variables. These variables were maternal contaminant concentration, maternal mass, maternal length, maternal blubber lipid, fetal length, fetal age (in Julian days), and fetal blubber lipid. Maternal age was available for 14 mother–fetus pairs, so this reduced data set was used to investigate the importance of female age. The objective of this analysis was to determine the set of best simple linear predictors of fetal contaminant levels that could be used in the future rather than assessing associations. The potential for collinearity existed between these independent variables, however, because some that were retained in the models following selection could still be significantly correlated with each other. Therefore, collinearity statistics in the form of variance inflation factors and condition indices were evaluated for all the variables in the models. Those with variance inflation factors of greater than five or condition indices of greater than 30 were excluded. In addition, only the individually significant variables were retained to produce the minimally simplest models for prediction that could be used in future analyses in which available data might be more limited. The intercepts,

Table 3. Mean ratios of contaminant concentrations for each fetus–mother pair with 2.5th and 97.5th percentiles[a]

	Premature			Late term		
	Mean ratio	95th percentiles	Sample size (*n*)	Mean ratio	95th percentiles	Sample size (*n*)
ΣPCBs	0.385	0.166, 0.882	9	0.512	0.138, 0.744	11
ΣPCB TEQs	0.408	0.132, 0.832	9	0.557	0.156, 1.121	11
ΣPCBs lipid wt	0.705	0.222, 1.025	9	1.186	0.613, 1.557	11
ΣPCB TEQs lipid wt	0.717	0.256, 0.991	9	1.295	0.585, 2.473	11
PCB 101	0.493	0.144, 1.060	9	0.507	0.180, 0.957	11
PCB 105	0.258	0.009, 0.526	6	0.473	0.154, 0.779	8
PCB 118	0.437	0.149, 1.056	9	0.521	0.168, 0.825	11
PCB 128	0.381	0.145, 0.673	9	0.525	0.160, 0.970	11
PCB 138	0.429	0.151, 0.843	8	0.540	0.135, 0.897	10
PCB 153	0.416	0.169, 0.827	9	0.536	0.536, 0.185	11
PCB 156	0.408		1	0.506	0.225, 0.651	7
PCB 170/194	0.234	0.105, 0.473	9	0.361	0.120, 0.660	10
PCB 180	0.277	0.115, 0.403	9	0.421	0.084, 0.704	10
PCB 190			0	0.153	0.104, 0.201	2
PCB 200			0	0.361	0.180, 0.513	5
ΣDDTs	0.461	0.140, 1.056	9	0.591	0.194, 0.967	11
ΣDDTs lipid wt	0.806	0.268, 1.359	9	1.383	0.722, 1.359	11
o,p′–DDD	0.415		1	0.498	0.258, 0.885	8
p,p′–DDD	0.583	0.183, 1.568	9	0.655	0.200, 1.154	11
p,p′–DDE	0.441	0.138, 1.050	9	0.596	0.191, 0.966	11
o,p′–DDT			1	0.645		1
p,p′–DDT	0.393	0.326, 0.430	5	0.590	0.097, 1.620	10

[a] Ratios were not calculated when samples were less than the limit of detection or when the compound could not be quantified because of an interferent. All ratios were calculated from wet-weight concentrations except where lipid weight (lipid wt) is indicated. TEQ = toxic equivalents; Σ = summed concentrations. DDD = dichlorodiphenyldichloroethane; DDE = dichlorodiphenyldichloroethylene.

coefficients, standard errors, *F* values, and *p* values from the final best-fitting models and set of variables for predicting fetal ΣPCBs, fetal ΣDDTs, and fetal TEQs are given in Table 4. All models displayed good fits to the data following inspection of the distribution of the residuals.

On a wet-weight basis (in both dependent and independent contaminant variables), maternal ΣPCBs explained 67% of the variance in fetal ΣPCBs, and fetal lipid explained another 29% (multiple $r^2 = 0.91$). Maternal blubber lipid also was significant, explaining a further 3%. The variance in lipid-weight fetal ΣPCBs was best explained by lipid-weight maternal ΣPCBs (56% of the variance explained) and maternal mass (17% of the variance explained; multiple $r^2 = 0.73$). Wet-weight maternal ΣDDTs (59%) and maternal age (37%) explained the greatest variance in wet-weight fetal ΣDDTs (multiple $r^2 = 0.72$). The variance in lipid-weight fetal ΣDDTs was explained largely by maternal lipid weight ΣDDTs (79%) and by maternal mass (19%; multiple $r^2 = 0.86$).

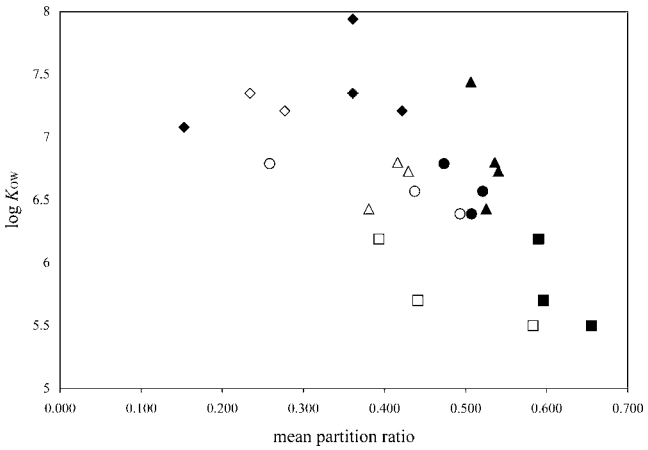

Fig. 1. Mean fetus to mother ratios for premature and late-term fetuses (Table 3) plotted against the logarithmic *n*-octanol/water partition coefficient (log K_{OW}). Values for K_{OW} of individual polychlorinated biphenyl congeners and DDT metabolites were obtained from the literature [41–43]. □ = premature *p,p′*-dichlorodiphenyldichloroethane (DDD), *p,p′*-dichlorodiphenyldichloroethylene (DDE), and *p,p′*-DDT; ■ = late-term *p,p′*-DDD, *p,p′*-DDE, and *p,p′*-DDT; ○ = premature pentachlorinated biphenyls; ● = late-term pentachlorinated biphenyls; △ = premature hexachlorinated biphenyls; ▲ = late-term hexachlorinated biphenyls; ◇ = premature heptachlorinated biphenyls; ◆ = late-term heptachlorinated biphenyls.

Finally, on a ΣPCB TEQ basis, fetal ΣPCB TEQ wet weight was explained by wet-weight maternal ΣPCB TEQ (69% of the variance) and fetal lipid (29% of the variance; multiple $r^2 = 0.83$). On a lipid-weight basis, this again simplified to only one predictor variable, maternal TEQ lipid weight, explaining 76% of the variance in fetal ΣPCB TEQ lipid weight.

Because the partition ratios suggest that fetal age plays a role in fetal contaminant levels, we investigated its role in the models described above. Fetal age and fetal length were positively correlated with fetal lipid (age: Pearson's $r = 0.504$, $p = 0.024$; length: Pearson's $r = 0.577$, $p = 0.008$), and the model selection process chose lipid as a better predictor. If fetal age and length are included in a stepwise model selection procedure without fetal lipid to predict fetal PCBs with maternal PCBs, then fetal age is retained in the model, although it is not individually significant ($p = 0.1396$).

In summary, robust linear predictions for all fetal contaminant concentrations in the blubber could be obtained using the concentration of contaminants in the mother's blubber, fetal and maternal blubber lipid content, and maternal mass and age.

DISCUSSION

The relationships between partition ratio, degree of chlorination, and log K_{OW} among the PCB congeners and DDT compounds support previous observations in laboratory animal

Table 4. Analysis of variance results from least-squares linear models for the best predictors of fetal contaminants (polychlorinated biphenyls [PCBs] and DDTs) on a wet-weight and lipid-weight basis[a]

Dependent variable	Predictor variables	Coefficient	Standard error	*F*	Pr(*F*)
Fetal ΣPCBs wet wt	Intercept	0.19	0.28		
	Maternal ΣPCBs wet wt	0.74	0.08	104.36	<0.0001
	Fetal blubber % lipid	0.03	0.00	46.06	<0.0001
	Maternal blubber % lipid	0.00	0.00	5.29	0.035
Fetal ΣPCBs lipid wt	Intercept	0.39	0.51		
	Maternal ΣPCBs lipid wt	0.70	0.11	35.1	<0.0001
	Maternal mass (kg)	0.01	0.00	10.9	0.0042
Fetal ΣDDTs wet wt	Intercept	1.31	0.73		
	Maternal ΣDDT wet wt	0.32	0.22	14.63	<0.003
	Maternal age (years)	0.10	0.03	9.17	0.01
Fetal ΣDDTs lipid wt	Intercept	0.06	0.42		
	Maternal ΣDDT lipid wt	0.77	0.08	83.59	<0.0001
	Maternal mass (kg)	0.01	0.00	20.22	0.0003
Fetal ΣPCB TEQs wet wt	Intercept	−0.28	0.17		
	Maternal ΣPCB TEQs wet wt	0.69	0.08	62.43	<0.0001
	Maternal age (years)	0.02	0.00	26.22	<0.0001
Fetal ΣPCB TEQs lipid wt	Intercept	0.70	0.17		
	Maternal ΣPCB TEQs, lipid wt	0.63	0.08	58.81	<0.0001

[a] All contaminant concentrations are reported as ng/g except for the toxic equivalents (TEQs), which are reported as pg/g. Σ = summed concentrations.

models that lower-molecular-weight, lipid-soluble chemicals accumulate more readily in the fetus compared with higher-molecular-weight, lipophilic compounds [15,46]. This variability may be explained by differences in blood transport mechanisms among the congeners, because individual congeners differ in their distribution in serum, erythrocytes, and lipoproteins (they are bound to lipoproteins and albumin rather than being dissolved in lipid) [47,48]. Interestingly, partition ratios for PCB compounds in maternal serum and umbilical cords in humans showed the opposite pattern. The mean ratio for pentachlorobiphenyls were 0.13, whereas those for hexa- and heptachlorobiphenyls were 0.18 and 0.23, respectively [49]. Although these studies are not directly comparable, because different tissues were sampled, this may reflect a difference in the effect of the placental barrier on OC transfer in sea lions compared to humans. California sea lions have a zonary, epitheliochorial placenta like other carnivores, whereas humans have a discoid, hemochorial placenta. These differences in placental shape and number of cell layers between fetal and maternal blood circulation also might affect the transfer of contaminants across the placental barrier. Differences in partition ratios are important, because different PCB congeners have different toxicological effects [50]. However, the effects on sea lion fetuses are still unknown. To our knowledge, data regarding the lactational transfer of OCs in California sea lions to determine the relative importance of transplacental and lactational transfer of the different PCB congeners and DDT compounds are not currently available for comparison. The lactational transfer of OCs in phocids is significant [8,10], and this route also likely is responsible for the bulk of maternal transfer of these compounds from mother to offspring in California sea lions. However, the duration and intensity of lactation in phocids (every day for weeks) and otariids (biweekly for months) differ greatly. Even so, because the transplacental route exposes the fetus to contaminants during early development, the potential for adverse effects on health of the offspring as a result of this earlier transfer may be significant.

Although the fetal blubber OC concentration was significantly related to the maternal blubber OC concentration, a number of other factors further influenced contaminant concentration in the fetus. Previous studies have shown that maternal blubber levels of OCs in pinnipeds are influenced by age, birth order (i.e., whether she was the first offspring of her mother), number of times she has given birth, and duration of lactation for each pup [1,9]. Blubber levels of ΣDDTs and ΣPCBs in pregnant sea lions in the present study were lower than those reported in recent studies of OC residues in California sea lions and were substantially lower than those reported in adult female sea lions sampled in the 1970s [1]. During the 1970s, mean ΣDDTs and ΣPCBs from the blubber of adult female California sea lions were approximately fourfold higher than the blubber concentrations reported here [21,51]. In animals sampled on San Miguel Island to investigate the etiology of premature parturition at that time, females that gave birth to premature pups were younger (age, eight years) and had significantly higher blubber OC levels compared with older females (age, 12 years) carrying pups to full term. Whether the OCs did, indeed, play a role in the etiology of the reproductive failure is uncertain, because the difference in OC levels between the two groups of sea lions could be explained by differences in the number of pups they had given birth to previously as well as the duration of gestation [22]. The results presented in the present paper show that the transplacental transfer of OCs in California sea lions is influenced not only by OCs in maternal blubber but also by maternal blubber lipid, mass, age, and stage of gestation.

Blubber levels of ΣPCBs in the present study also were lower than recent results reported by Lieberg-Clark et al. [52], Kannan et al. [53], and Ylitalo et al. [23]. The difference between OC levels reported here and those in the Lieberg-Clark study may be explained by sex differences in the animals sampled. All animals in the Lieberg-Clark study were subadult and adult males, which do not depurate their contaminant load in an annual reproductive event. In addition, differences between the two studies may result from different analytical methods used to measure the OCs and lipid content. Differences among levels of contaminants in the present study and those in the studies by Kannan et al. [53] and Ylitalo et al. [23] might be explained by the health status of the sea lions. All the animals in the present study were in good body con-

dition and were either sampled alive or shortly after dying from acute domoic acid toxicosis. The studies by Kannan et al. [53] and Ylitalo et al. [23] included sea lions with chronic disease, such as carcinoma. Blubber lipid concentration has a marked influence on OC concentrations; furthermore, animals dying from carcinoma had blubber concentrations of ΣPCBs (based on wet wt) almost twice those in sea lions that died from other causes and concentrations of ΣDDTs (based on wet wt) that were approximately 30% higher [23].

All adult females sampled in the present study appeared to be in good body condition (clinically healthy and good body wt), but some of the blubber samples had low lipid content. The three lowest lipid values obtained from adult females were from animals that were sampled while alive. This is consistent with results from live, apparently healthy harbor seals that also exhibited a low lipid content (7%) [54], and it suggests that both blubber depth and lipid content should be considered when evaluating nutritive state.

In conclusion, these findings demonstrate that OCs are transferred from mother to fetus in the California sea lion. In addition, the transfer of OCs varies with stage of gestation, with premature fetuses showing lower partition ratios than late-term fetuses that are approaching steady state with the mother. Blubber concentrations in the fetus are predicted by maternal concentrations, maternal mass and age, as well as lipid content of the blubber in both the mother and fetus, and they likely are influenced by congener or compound structure. Data obtained for these intrinsic factors in follow-up monitoring studies will enable fetal concentrations to be estimated with relative accuracy in the future. It is rare to be able to obtain paired maternal and fetal blubber samples that have not undergone autolysis, as reflected by the paucity of published studies in this area. Population-level effects can be estimated only if information regarding the transfer of compounds at different life stages is available together with associated levels of uncertainty.

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